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Submitting Samples for Fish Disease Diagnosis

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Submitting Samples For Fish Disease Diagnosis

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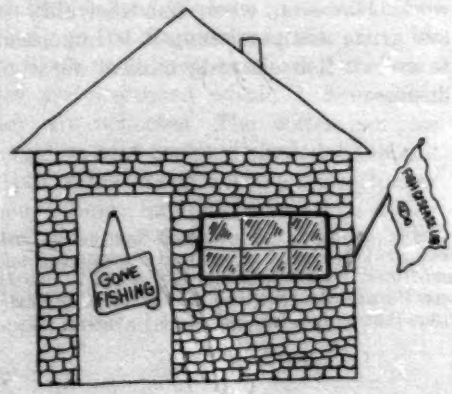
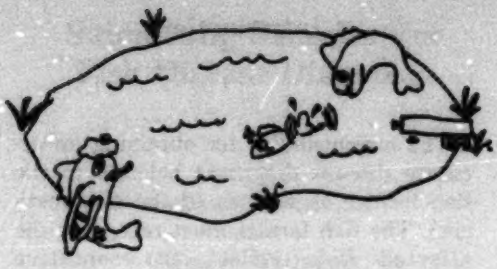
Introduction

The responsibility for obtaining an accurate disease diagnosis belongs to the fish farmer as well as to the diagnostician. The fish farmer must recognize the affected fish, collect representative specimens, prepare them properly for shipment, and use the best mode of transportation available. He must also provide a water sample and adequate information about water quality, culture techniques, and numerous other factors that could affect fish. Failure in any of these steps can result in an inaccurate or incomplete diagnosis. To avoid frustration, loss of time, and unnecessary expense, *the farmer should telephone the diagnostician* before any specimens are collected, to be sure that the sample will be processed immediately upon arrival.





Collection of Fish and Water Samples

The likelihood of a sound diagnosis is highest if 3 to 10 sick or dying fish are provided. Dead fish are usually unsuitable unless pesticide kills are suspected.[1] If the fish collected for the diagnostic sample are severely affected, additional fish should be added for replacement of possible losses during transport. If a disease problem is severe, it is likely that all fish in a pond are affected and that any of them would be suitable for diagnostic work. However, when fish mortality is low grade and persistent, it is important to collect fish showing clinical signs of disease.

[1]Living or dead fish collected from suspected pesticide kills should be wrapped in aluminum foil and frozen. Such fish should not be put in plastic bags because the bags may release traces of plasticizers that confuse the analysis.

Usually, sick fish can be picked up around the edges of the pond, by hand or with a dip net or cast net. The farmer should not use fish caught with hook and line or baited and trapped with feed, because these fish will probably be healthy, or among the least affected individuals. Random seining or the use of a throw or cast net is sometimes effective for catching diseased fish. The diagnostic sample should be collected before any treatments are begun and should be transported to the diagnostician immediately. A delay of several hours can change the disease picture and confuse the diagnosis.

A water sample should be collected, at least 50 feet from an inlet or drain, in a clean glass jar about 1 quart in size. The empty jar (with lid tightly fastened) should be submerged to a depth of about 1 foot in water about 5 feet from the pond edge. The cap should then be removed and inverted so that all air is expelled from it as well as from the jar. The jar should be tightly recapped while it is still under water. As soon as the water sample has been taken, it is best to take it immediately to the diagnostic facility. Results are likely to be most accurate if the samples are processed within 1 hour after they are collected. The water samples should be kept cool and shaded, or should be put on ice if they will be en route longer than 1 hour. Samples more than a few hours old are of little value, although they can be used to determine total alkalinity, a measure necessary for safe and effective copper sulfate treatments.

Information Needed By Diagnosticians

To provide an accurate diagnosis, the diagnostician must have information about the fish, water, surrounding facilities, and cultural practices. When possible, the sample should be brought to the diagnostician by a person who is knowledgeable about the facility and the disease problem, and is thus able to provide any additional information needed.

The first information needed by the diagnostician is the name of the fish producer and of his business, together with address and telephone number. Also very important in diagnosis and treatment are the name or number and type (e.g., pond, raceway, tank) of the water body, along with the water source (e.g., well, surface runoff, or another pond), surface area, average depth, water temperature, water chemistry (total alkalinity, pH, nitrite, unionized ammonia, carbon dioxide, and oxygen), color, odor, depth of visibility, and presence or absence of scum or moss. Fish age, size, and

original source, along with standing crop (density), are also important. The disease history of the affected and surrounding ponds, along with information on previous treatments—including weed treatments—should be supplied. Information concerning agricultural activities around the pond, particularly the spraying of agricultural chemicals, is needed. The fish producer should also inform the diagnostician when the pond was last drained or seined, when water or fish were added, the type and rate of feeding, type and application rate of fertilizer used, of an occurrence of low oxygen, and of inclement weather conditions. Information is also needed on how and when the water and fish samples were collected.

Information on the number of fish that have died, the daily loss, and the duration of the die-off is helpful because some diseases follow definitive mortality patterns, knowledge of which can aid the diagnostician. Any sick or dying turtles, frogs, tadpoles, crayfish, ducks, snakes, trash fish, or other wildlife should be reported. The farmer should mention to the diagnostician any unusual signs observed in sick, dying, and dead fish. Fish captured and

brought in may not show the same signs that the farmer has been seeing, but his description may enable the diagnostician to reach a better understanding of the condition.

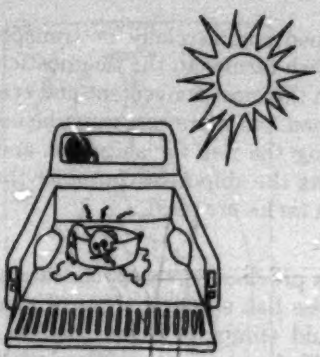
Some common disease signs follow: redness of the skin, fins, belly or side; sores, ulcers; dull or discolored areas; bloated belly; popeyes; frayed and eroded fins; raised, loosened, or missing scales; erosion of mouth or barbels; emaciation; air bubbles under the skin, in eyes, or in fins; pale or dark color; broken-back condition; opaque eyes; cysts in or on the skin; excess or absence of mucus covering the skin; bright red, dark red, pale, brown, frayed, uneven, or puffed gills; gills with decayed or discolored areas on body; bloody or discolored internal organs; and fluid-filled intestine or body cavity.

Some common behavioral signs include topping; crowding of fish near the bank, inlet, or aeration devices; erratic swimming; listlessness; spiral swimming; flashing; and lying on the bottom.

Transporting Affected Fish

It is important to provide living fish to the diagnostician. The delivery of dead specimens, and especially decayed material, may merely waste time and delay corrective therapy. Four transportation methods are discussed here.

1. In water in open containers. Diseased fish are already stressed, and their ability



to survive is impaired; consequently the fish should be held at the lowest practicable density and provided the best possible care. Neither the fish nor water should be treated, however, because treatment may interfere with the diagnosis. Live specimens may be transported in tanks, tubs, or buckets with plenty of water. When small numbers of fish are being transported for short distances, supplemental aeration is not normally required, provided the water is kept cool and the vehicle remains in motion. More aeration is provided by sloshing the water in tanks and tubs with a large surface area than in buckets or other small containers. When large numbers of fish are transported, or the transport period is long, supplemental aeration with agitators, compressed air, or bottled oxygen is essential. An insulated container might be required for fish transported in the back of an open pickup truck during summer or winter.

2. **In plastic bags.** Live fish can be placed in water-filled plastic bags in which air is replaced with oxygen, and

shipped commercially or transported by the fish farmer to the diagnostician. It is often the most convenient and economical method, but more care must be used in selecting the fish for shipment and in preparing the shipment container than when open tanks are used.

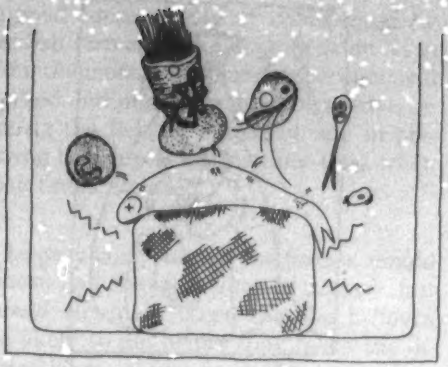
As previously stated, the farmer should choose fish showing signs of disease, but should eliminate those that he believes are likely to die before they reach the diagnostician. A few dead fish in the bag may foul the water and kill the others. The smaller affected fish should be selected, because they require less oxygen and water than large fish. Survival in shipping is enhanced by keeping the fish cool—often by placing ice in the shipping container, but outside the plastic bag. The quickest, most reliable transportation should be used.

Fish should be placed in doubled heavy-strength plastic bags that can be sealed water- and air-tight, and filled from one-sixth to one-fourth of their volume with chlorine-free water. Density of fish in the bag should be low. No chemicals should be added to the water. The air should be pressed from the bag, and the displaced volume filled with oxygen. Strong rubber bands can be used to tie off the top, and bags can then be placed in insulated boxes of suitable size. If the farmer is transporting the fish directly to the diagnostician, the bags can be carried in the passenger compartment to avoid temperature extremes.

3. On ice or frozen. Fish should be transported on ice or frozen when it is not pos-

sible to get live fish to the diagnostician. When the fish can be transported immediately and the time en route is short, fish should be put in a plastic bag without water and placed on ice. If the ice is likely to melt during transport, an insulated container can be used. The diagnostician may be misled by the signs and parasite load of fish transported on ice without the protection of a plastic bag. Parasites—particularly protozoans—often detach themselves from iced fish, so that the parasite load, which often must be known to help determine cause, cannot be ascertained. Also, fish often hemorrhage at the fin bases or wherever they contact the ice, giving false disease signs.

When the distance is great or the fish cannot be transported immediately by the farmer, they should be put in a doubled plastic bag with a small quantity of water (enough to cover the fish), sealed water-tight, and frozen solid overnight. Frozen fish should be placed in a well-insulated container to prolong the



frozen state. Ice does not always keep the bagged fish frozen for more than 24 hours. However, they can usually be kept frozen for 48 hours or longer if the bags are put in a small (less than 1 cubic foot), well-insulated container with either 5 to 10 pounds of dry ice or two or three large artificial ice packs. Dry ice should be wrapped separately in a paper bag, but should not be put in an air-tight container. Fish should be shipped as soon as possible after freezing and should not be held more than a month in a freezer unless they are being saved for pesticide analysis.

4. Chemically preserved. Sometimes the only method of getting fish to the diagnostician is by shipping chemically preserved specimens. When fish are fixed (tissue components are denatured so the tissues can be held in preservation) and then preserved, they can be checked only for certain parasites and the presence or absence of certain bacteria. A 10% for-

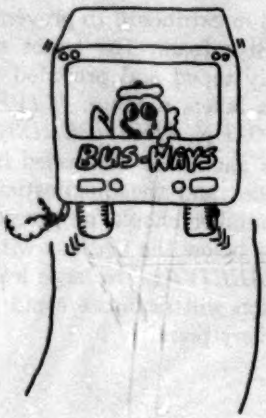
malin solution (one part of 37–40% formaldehyde to nine parts of water) is the best general fixative and preservative. Alcohol (70%) is a useful preservative, but dehydrates many parasites and makes them more difficult to identify. Only live whole fish or tissues removed from freshly killed fish should be preserved because fish dead for only 1 hour may be useless to the diagnostician. The samples should be placed in the fixative as rapidly as possible, since the tissues and many disease pathogens start to break down immediately after death. If the fish are longer than 1½ inches, the body cavity should be slit to allow immediate penetration of the fixative. Affected portions of fish too large to send whole should be cut into pieces appropriately sized for shipping, before fixation. In removing samples of affected tissues, the farmer should excise a section near the edge of the lesion that includes both affected and non-affected tissue. At least five times more fixative than tissue should be used. After 24 hours in fixation, a much smaller ratio (about 1:1) of preservative to tissue can be maintained.

Once the affected fish or fish parts are fixed, they can be shipped. For best shipping results, paper towels are dampened with the preservative, and the towels and fixed specimens are placed in a sealable double plastic bag and sent in a sturdy box. This method avoids the breaking of glass and spilling of preservative en route. If a glass bottle must be used, it should be wrapped in a plastic bag that can be sealed, and packed well to prevent breakage. Preserved specimens can be shipped by Parcel Post or United Parcel Service.

Care should be used in handling all fixatives and preservatives because they are strong irritants to eyes, mucous membranes, and skin, and are poisonous if swallowed.

Commercial Shipping Of Fish Specimens

Frozen or live fish not transported by the fish producer should be sent by the fastest and most reliable commercial method available. Direct air shipment is usually the method of choice, but changes in airlines or planes between origin and destination can cause delay or loss. Special express delivery or courier service airlines guarantee 24-hour delivery anywhere in the United States, but such service is expensive. For shipping to many diagnostic laboratories, bus lines are often best because the laboratories are likely to be close to bus stations but may



be many miles from the nearest major airport. Bus service can be efficient, providing less-than-24-hour service to most places in the Southeast. When using bus service, one should try to use the same bus line from origin to destination. Changing lines can cause delay. Express delivery is the most reliable bus service because it guarantees that the package will be on the next bus out whenever there is a bus change at a terminal, and the cost is usually only about half that of air service. United Parcel Service has often been used for shipments of fish, but because about 48 to 72 hours are required for most deliveries, sick fish may die or frozen samples may spoil. Other express delivery services may be available, but are usually expensive.

The sample must be packed carefully and adequately. Styrofoam containers should be put in cardboard boxes or

wrapped in cardboard to prevent damage to the Styrofoam. The boxes should be throughly taped and provided with conspicuous labels reading, "*LIVE FISH*," or "*FROZEN FISH (PERISHABLE)*." The box should be addressed in at least two places and the diagnostician's telephone number should be placed in large numbers below the address with "*CALL UPON ARRIVAL*" in large letters. This precaution will facilitate rapid pickup at the delivery point.

Illustrations by Gaye Hiryak

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.